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Parturition and the Perinatal Period: Can Mode of Delivery Impact on the Future Health of the Neonate?

Special Issue "Advances, challenges, and controversies in perinatal physiology"

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Key Words

Pregnancy, caesarean section, infant, health, microbiome, immunity, lifecourse, vaginal delivery, stress hormones, epigenetics

Biography



Dr Rachel Tribe, PhD and Dr Paul Taylor, PhD (pictured above) are physiologists who undertake translational research focussed on improving outcome for pregnant women and their children. Senior academics in the Dept. of Women and Children's Health, King's College, they both run thriving research groups and publish widely on pregnancy complications and the impact of pregnancy on life-

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long health. Niamh Kelly has recently joined Dr Tribe's team to study breastfeeding, probiotics and infant health. Doug Rees is a PhD student in Dr Taylor's team studying the gut microbiome in obese pregnancy. Professors Jane Sandall (King's College London) and Holly Kennedy (Yale School of Nursing) have academic interests in birth outcomes, epigenetic changes during labour, and clinical care factors to optimise birth.

Abstract

Caesarean section and instrumental delivery rates are increasing in many parts of the world due to a range of cultural and medical reasons, with limited consideration as to how 'mode of delivery' may impact on childhood and long-term health.

However, babies born particularly by pre-labour caesarean section appear to have a subtly different physiology to those born by normal vaginal delivery, with both acute and chronic complications such as respiratory and other morbidities being apparent. It has been hypothesised that inherent mechanisms within the process of labour and vaginal delivery, far from being a passive mechanical process by which the fetus and placenta are expelled from the birth canal, may trigger certain protective developmental processes permissive for normal immunological and physiological development of the fetus postnatally. Traditionally the primary candidate mechanism has been the hormonal surges or stress response associated with labour and vaginal delivery, but there is increasing awareness that transfer of the maternal microbiome to the infant during parturition and transgenerational transmission of disease traits through epigenetics are also important. Interventions such as probiotics, neonatal gut seeding and different approaches to clinical care have potential to influence parturition physiology and improve outcomes for infants.

Introduction

Given the fundamental nature of parturition, there is relatively limited understanding of how 'the route by which a baby is born' can have prolonged physiological consequences for infants. The process of normal parturition (labour) can take many hours, and a neonate can be exposed to a range of mechanical forces, periods of transient hypoxia, oxidative stress, and

stress hormone surges that can potentially influence physiology and impact the life-long health of the baby. However, the mechanisms responsible for transmitting these physiological stimuli have yet to be fully elucidated.

Now, more than ever, a greater understanding of how mode of delivery (and onset, progression, and duration of labour) can impact on normal development and long-term health outcomes for the baby is critical given the increasing caesarean section (CS) rate worldwide.

A growing number of studies indicate that babies born by pre-labour (elective) CS appear to have a subtly different physiology to those born by normal vaginal delivery (VD), with both acute and chronic complications such as respiratory and other morbidities being apparent (Hyde & Modi, 2012; Taylor, 2015; Dahlen *et al.*, 2016). It has been hypothesised that labour and VD, far from being mere passive mechanical processes by which the fetus and placenta are expelled from the uterus, provide important, permissive developmental cues required to trigger immunological and physiological maturation of the fetus in preparation for postnatal life.

This review examines the most recent epidemiological and experimental evidence linking mode of delivery and future outcomes in infants and discuss the three main mechanisms that have been proposed to explain why mode of delivery, spontaneous and/or induced/augmented vaginal delivery *versus* caesarean section, may affect neonatal development. These are (1) exposure to different levels of physical stress and stress hormone surges during delivery (2) aberrant microbial colonisation of the infant intestinal tract; and (3) epigenetic modification of gene expression. The scientific evidence in support of (or contraindicating) clinical interventions such as bacterial ‘seeding’ of the neonatal gut microbiome via vaginal swabs and probiotic supplementation is also considered. Finally, we will discuss potential clinical care factors that could optimise physiological processes during parturition.

What is the evidence that mode of delivery impacts on infant health and immune system?

Approximately, 1 in 5 women around the world will give birth via CS; and there have been repeated reports of increasing CS over time (Betrán *et al.*, 2016; Miller *et al.*, 2016). CS rates in England are now approximately 28%, and even higher in the USA (32%) and Brazil

(51.9%) (NHS Digital, 2017; Martin *et al.*, 2015; Nakamura-Pereira *et al.*, 2016; Miller *et al.*, 2016). This rise predominantly parallels the rapid increases in maternal obesity, macrosomia and pregnancy complications such as pre-eclampsia, and a concomitant increase in elective CS. A rise in CS rates does not necessarily correlate with improved long term outcomes (Miller *et al.*, 2016), as increased maternal request for CS can be due to a range of social and cultural pressures, fear of pain; the belief that CS is safer for the baby; convenience for health professionals, mothers and family; fear of medical litigation and lower tolerance to any complications or outcomes other than the perfect baby.

In the UK, there is consensus that elective CS should only be offered in the context of providing women with balanced information of the risks and benefits (UK National Institute for Health and Care Excellence, Caesarean Section Clinical Guideline CG132 <https://www.nice.org.uk/guidance/cg132/>). However, the World Health Organisation has stated that there is no evidence to suggest a mother or child would benefit from performing a CS when not medically indicated (World Health Organisation, 2015) and recommend further research into the effects of CS on outcomes such as maternal and perinatal morbidity, paediatric outcomes, and psychological and social well-being.

Strong epidemiological evidence supports the concept that CS is associated with both short and long-term health effects via altered immune system and metabolism (Hyde & Modi, 2012; Taylor, 2015; Dahlen *et al.*, 2016). Infants delivered via CS are more likely to develop respiratory and immune disorders such as asthma, atopy, juvenile arthritis and inflammatory bowel disease (Bager *et al.*, 2008; Sevelsted *et al.*, 2015). Interestingly, the effects of CS can vary between elective pre-labour CS and emergency CS when a woman is in established labour; this provides a natural control for differences in some neonatal exposures. In a register-based cohort study of 750,000 children born in Denmark (1997-2013), risk of lower respiratory infection and asthma is higher in elective CS than emergency CS (Kristensen & Henriksen, 2016). The Prediction of Allergy in Taiwanese Children (PATCH) study, a prospective birth cohort of 579 children, reported that CS was associated with an increased incidence of 'wheeze' and reduced lung function at 12 months of age (Liao *et al.*, 2017). Furthermore, these infants had impaired neonatal production of pro-inflammatory cytokines in isolated mononuclear cells linking mode of delivery with development of the neonatal immune system (Chu *et al.*, 2017b; Liao *et al.*, 2017). The longer term effects of caesarean section on wheeze and allergy have been explored in a metanalysis; there was an increased

odds ratio for asthma up to 12 years of age, with less conclusive results for wheeze and allergy/atopy (Keag *et al.*, 2018).

Early experimental suggests that predisposition to immune disorders may be due to altered immune maturation and reduced exposure to the maternal microbiota (see section below) in infants delivered via CS (Daniel *et al.*, 2008; Cho & Norman, 2013; Thysen *et al.*, 2015; Treviño-Garza *et al.*, 2016). In an animal model of CS, Daniels *et al.* (2008) found that piglets born by caesarean had increased basal serum levels of TNF- α at 2 weeks of age, while interferon (INF- γ) was lower. Some variations in immune function in infants delivered via CS rectify themselves over time, with basal serum levels of adrenocorticotrophin and cortisol being elevated at birth in piglets born via CS but did not differ from VD piglets at 2 weeks. Thus, long-term alterations in immune maturation in CS infants should be examined.

Higher rates of overweight and obesity have been observed in individuals born via CS (Yuan *et al.*, 2016; Pluymen *et al.*, 2016; Keag *et al.*, 2018). These effects can extend into adulthood, increasing the risk of developing metabolic syndrome and cardiovascular disease (Hyde & Modi, 2012; Darmasseelane *et al.*, 2014). Data from a large US cohort, the ‘Growing Up Today Study’ (GUTS, 22,068 individuals aged 9-14 years and 20-28 years) found, after adjusting for confounding factors, that any CS was associated with 15% increase risk of obesity, while an elective CS was associated with an ever higher (30%) increased risk (Yuan *et al.*, 2016). CS may also be an independent risk factor for adult hypertension (Fernandes *et al.*, 2015).

CS can also impact on neurological outcomes including schizophrenia, autism spectrum disorders (ASD), altered longer-term dopamine responses and behaviour (Boksa & El-Khodor, 2003; Curran *et al.*, 2015b). The concept stemmed from an interesting series of animal studies on CS (+/- anoxia exposure post birth) (Boksa & El-Khodor, 2003) and has promoted an ongoing debate as to whether childhood emotional and behavioural problems may be increased in children delivered by CS. However, whilst associations have been identified between CS ASD, attention deficit hyperactivity disorder (ADHD) (Curran *et al.*, 2015b) and behavioural problems (Silva *et al.*, 2014), other studies using similar methodologies could not replicate the findings (Khalaf *et al.*, 2015; Curran *et al.*, 2016a, 2016b). Curran and colleagues, following a systematic review and meta-analysis of the literature (Curran *et al.*, 2015b), undertook analyses of sibling pairs discordant for ASD and

ADHD and revised their conclusions. Whilst children from the Swedish Medical Birth Register born by CS were approximately 20% more likely to be diagnosed as having ASD, the association did not persist when employing sibling controls, implying that the association was due to familial confounding by genetic and/or environmental factors (Curran *et al.*, 2015a). Similarly, when investigating mode of delivery and ADHD in sibling pairs, CS was associated with a small increased risk of ADHD; however, among siblings the association only remained for emergency CS and not elective. If this were a causal effect, the association would be expected to persist for both types of CS (Curran *et al.*, 2016b).

Potentially, there may also be associations with altered childhood outcomes with more mechanical (vacuum and forceps) modes of delivery and pharmacological interventions as well as exposure to antibiotics in the intrapartum period, but this requires more research. Indeed, the Epigenetic Impact of Childbirth (EPIIC) hypothesis proposes that interventions in labour such as exposure to synthetic oxytocin during labour induction and augmentation could influence neonatal health through mediation of epigenetic changes and there are some interesting data from animal studies (Dahlen *et al.*, 2013; Boksa *et al.*, 2015) suggesting that maternal oxytocin administration may have multiple acute effects on central nervous system metabolic responses to anoxia at birth involving oxidative stress and inflammation which can affect the molecular epigenetic machinery.

Differences in the physiological impact of vaginal *versus* caesarean section delivery

Interruption of the normal parturition physiological pathway in CS

There are clearly differences in the timing and physical experience of vaginal delivery *versus* elective CS, but the question is what are they and why do they matter? The first consideration is that neonates delivered prior to labour onset even at term gestations (i.e. elective CS), have not been exposed to the full gamut of hormonal and inflammatory signals associated with parturition. Depending on the gestation age at which elective sections are performed this could *i)* reduce exposure to physiologically increasing concentrations of hormones such as oestrogen and cortisol, *ii)* the effects of functional progesterone withdrawal and *iii)* sub-optimal preparation for neonatal transmission (e.g. maturation of fetal lung, fluid absorption mechanisms and exposure to surfactant proteins). The latter, in part contributes to

the association between CS and childhood lung function (Ramachandrappa & Jain, 2008). However, in many epidemiological studies, gestational age at time of elective CS has not always been controlled for or indeed distinction made between elective *versus* emergency CS and prior exposure to induction/augmentation agents such as prostaglandins and oxytocin.

There is accumulating evidence that labour influences hypothalamic–pituitary function at birth. Umbilical venous plasma prolactin levels are significantly lower in pre-labour CS neonates (Heasman *et al.*, 1997). Plasma concentration of thyroxine and triiodothyronine and cortisol concentration are also lower after pre-labour CS compared to labour, with higher mean umbilical plasma thyroid-stimulating hormone (TSH) concentration (Bird *et al.*, 1996). Labour reduces plasma TSH concentrations at birth in association with a surge in cortisol and appears to be a developmental stimuli in the subsequent surge in triiodothyronine (T3) that occurs postpartum after vaginal birth (Bird *et al.*, 1996). The consequences of an absent T3 surge at birth is not entirely clear, but T3 has important maturational effects in the fetus, and in congenitally hypothyroid rats, early postnatal (P7) but not late (P21) T4 treatment improves learning and memory performance (Reid *et al.*, 2007) supporting a neurotrophic role of thyroid hormones during certain critical developmental windows.

Umbilical cord concentrations of adrenaline, noradrenaline, and cortisol are also significantly lower in babies born by CS compared with VD (Vogl *et al.*, 2006), which may result in impaired development of the HPA axis and abnormal reactivity to stress (Taylor *et al.*, 2000). The absence of stress hormones in the cord blood, may cause maladaptation of the HPA axis and may also explain why babies delivered by elective and emergency CS have lower systolic blood pressure on the first day of life. This may also be due to the use of anaesthetics during surgery, but clearly these early haemodynamic changes may have implications for the plasticity of neonatal cardiovascular and autonomic nervous system development (Sedaghat *et al.*, 2008). Maladaptations made during birth may establish physiological set points for a developmental trajectory for future cardiovascular dysfunction and disease. Heart rate variability analysis from ECG traces would allow assessment of the relative contribution of the sympathetic and parasympathetic components of the autonomic nervous system in the regulation of blood pressure (BP), for which sympathetic tone is a biomarker for subsequent cardiovascular disease. The contribution of anaesthetic regimens employed during CS is not easily dissected, although well designed animal studies provide proof of principle and invaluable mechanistic insight in this regard.

Physical forces and stress responses to labour versus CS

A second consideration are the physical forces a fetus experiences during labour. Early labour and established labour can manifest for many hours (and even days). This exposure to intermittent contractile forces and intermittent hypoxia and oxidative stress (due to rhythmic spontaneous contractions) as well as other hormonal responses to maternal stress and pain have significant potential to influence physiological development of the neonate (Lagercrantz, 2016). The physical forces exerted through the second stage are also important – in response to strong propulsive contractions generated by the uterine fundus, the fetus is squeezed through the cervical opening and upwards through the pelvis and out through the vaginal canal. A fetus born via CS experiences very different forces and pressures and is delivered far faster than a vaginal birth. Indeed, delivery seems to be a necessary stress, which acts as a cue for the development of various systems in the infant.

The physiological importance of the process of delivery is also exemplified in foals presenting with neonatal equine maladjustment syndrome, or “dummy foal” syndrome. This condition, associated with rapid assisted delivery, is characterized by low awareness, abnormal sucking and inability to bond with the mare. It is thought that this is caused by the absence of major physiological milestones during delivery (Aleman *et al.*, 2017). Levels of inhibitory neuro-steroids are elevated in these foals after birth, keeping them in a reduced state of consciousness (Aleman *et al.*, 2017). The ‘Madigan squeeze’ technique, used to treat these foals, involves compressions of the thorax for 20 minutes, mimicking compression of the fetus in the birth canal during normal delivery. Foals that receive this treatment recover faster than those that receive medication only (Aleman *et al.*, 2017).

The mode of delivery can also affect steroids concentrations in neonates. McCallie *et al.* (2017) measured the natural course of nine neuro-steroids over the first 2 days of life in 39 full-term neonates. The postnatal decline in progesterone, epipregnanolone and pregnanolone-sulphate was more significant in infants born by VD compared to pre-labour CS (McCallie *et al.*, 2017). As this reaction is similar to that of foals with neonatal equine maladjustment syndrome, the authors suggest that this may be due to a lack of labour rather than the delivery mode itself. The speed at which elective CS are carried out, and the lack of the physical forces of labour, may skip vital steps needed to prepare the fetus for extrauterine transition. Lack of ‘vaginal squeeze’ had been investigated in terms of poor neonatal lung

clearance at birth (Jain & Eaton, 2006), but the consensus now holds that reduced ENaC channel expression/function, secondary to lower levels of corticosteroid (or stimulatory catecholamines) at birth is a more plausible explanation, although these mechanisms need not be mutually exclusive.

Yektaei-Karin and colleagues (2007) speculated that the reduced cortisol surge, measured in cord blood, associated with CS (Gitau *et al.*, 2001) may underpin differences in immune function observed with VD *versus* CS. The detected rise in IL-8 and neutrophils and monocytes in normal labour was suppressed in CS infants and exaggerated in assisted delivery. They proposed that the setting of the HPA-axis at birth is a promoter of an alarm response and a surge of neuroendocrine immuno-modulating factors. Suppression of antimicrobial defences in the new-born delivered by CS, could have profound implications for immune function development in the neonate.

The reduced exposure to the physical factors and stress hormones in CS compared with VD also results in a significantly increased infant response to stress/pain two hours after birth (Chiş *et al.*, 2017), indicative of altered programming of the HPA axis. Whether this difference was directly linked to stress of delivery or type of anaesthesia could not be determined, but the altered stress responses seems to remain into childhood as children born via CS are more likely to suffer from anxiety, depression and sleep disturbances (Kelmanson, 2013).

Animal models of CS and ‘stress’

Animal models are needed to fully elucidate the complex mechanisms implied by the observational human studies described above. There are still many gaps in the literature especially for longer term cardiovascular outcomes. In terms of neurodevelopment, it has been found in rats that CS can produce long-term changes in central nervous system dopamine signalling, compared to VD (Boksa & Zhang, 2008). Similarly, rat pups born by CS demonstrate long-term reciprocal changes in dopamine (DA) levels and metabolism in the nucleus accumbens and prefrontal cortex (El-Khodori & Boksa, 1997). Adrenaline administration to rats at birth following CS prevents these long-term neurological changes as well as the observed increase in tyrosine hydroxylase (TH) activity in response to a stress challenge in adulthood (Boksa & Zhang, 2008).

Another rodent model of mode of delivery, employed CS delivery in guillotined rat dams, to dissect the influence of isoflurane/N₂O anaesthetic effects and global birth anoxia on brain biochemistry in neonatal rats compared with controls (Vaillancourt *et al.*, 1999). A short period of global anoxia just before birth, increased blood and brain lactate levels, decreased brain ATP and increased systemic PO₂, presumably due to ventilatory responses to hypoxemia and hypercapnia (Vaillancourt *et al.*, 1999). Animal studies also indicate how hypoxia at birth can contribute to long-term changes in brain chemistry and behaviour (Venerosi *et al.*, 2006).

Overall, the lack of exposure to physical forces of labour and associated stress hormones appears likely to have an influential effect on neonatal outcomes. Longer term follow-up will determine the persistence of these phenotypic changes into childhood and beyond.

Impaired microbial colonisation of the infant intestinal tract

Mode of delivery can determine the bacterial populations which colonise the infant microbiota in the first day of life (Dominguez-Bello *et al.*, 2010; Bäckhed *et al.*, 2015; Bokulich *et al.*, 2016; Azad *et al.*, 2016; Yassour *et al.*, 2016; Sakwinska *et al.*, 2017; Chu *et al.*, 2017a). Common practices in the delivery theatre, including intravenous prophylactic antibiotics up to one hour before surgery, chlorohexidine alcohol preparation of the skin and hair removal, and removal of maternal stool contamination in the birthing area, all impact the bacterial exposure of the infant (Azad *et al.*, 2016; Martinez *et al.*, 2017), as well as CS effectively bypasses normal microbial seeding by maternal vaginal and intestinal flora.

Exposure to the vaginal microbiota appears to be important for initial colonisation of infant skin and gut. Infants born naturally via vaginal birth possess undifferentiated bacterial communities on their skin, oral and nasopharyngeal cavities, and gut, that resemble the maternal vaginal microbiota. In contrast, CS infants have an initial microbiota more closely related to the maternal skin microbiota (Dominguez-Bello *et al.*, 2010; Jakobsson *et al.*, 2014). Importantly, CS infants have lower levels of the protective *Bifidobacterium longum* sub species *infantis* (*B. infantis*) at just three days olds (Biasucci *et al.*, 2010). More recent evidence indicates that exposure to maternal rectal bacteria during VD may be more important than the vaginal microbiome in the vertical transmission and colonisation

(‘seeding’) of the infant gut (Sakwinska *et al.*, 2017). Indeed, it is more plausible that infant gut colonisation should be driven by transfer of maternal intestinal microbiota rather than vaginal bacteria given the very different environments these species needed to thrive. There are also data suggesting microbial transfer from breast milk (Pannaraj *et al.*, 2017). These routes of transmission still require more investigation as although Sakwinska and colleagues (2017) reported a greater similarity between paired maternal rectal and infant stool microbiota, it was minimal in early infancy and only showed a strong resemblance 4 to 6 month after birth. They concluded that physiological conditions such as immune system maturation, which could also be affected by mode of delivery, could be important. It was also not discussed whether all VD babies were exposed to maternal stool bacteria during birth.

Clinical confounders such as onset of lactogenesis can be affected by labour interventions and are not always considered by studies linking mode of delivery and the gut microbiome. Breastfeeding can be challenging for women who have had a CS. Duration of breast feeding can be curtailed and there can be an initial physiological delay in the onset of lactogenesis (Hobbs *et al.*, 2016), both situations that could have a direct impact on microbiota development, i.e. Bifidobacterial colonisation is dependent on presence of human milk oligosaccharides in the breast milk, (Underwood *et al.*, 2015). Decreased numbers of intestinal Bifidobacteria have clinical relevance as this type of intestinal dysbiosis early in life predisposes to inflammation (Sirilun *et al.*, 2015; Walker, 2017) and numerous studies have observed positive associations with bifidobacterial strains and normal neonatal gut microbiota (Guo *et al.*, 2015; Patole *et al.*, 2016; Stewart *et al.*, 2016, 2017)

Similarly, augmentation of labour with oxytocin, maternal obesity and increased maternal age can impact on establishment of breastfeeding and breast-feeding duration. the link between mode of delivery and gut microbiome in infants, 6 weeks post birth, was recently refuted in a small cohort of 81 women (Chu *et al.*, 2017a). In contrast to others, this study attempted to control for several fixed effects including intrapartum antibiotics, breastfeeding practices, gestational weight gain, maternal gestational diabetes or pre-pregnancy BMI, and gestational age at the time of delivery. However, the sample size was relatively small and significance was borderline.

The causal link between dysbiosis at birth/the early neonatal period and future human health needs more supporting evidence. The mechanistic pathways have been examined in some detail in animal models (see below, Figure 1) and strengthen the argument for a causal relationship between gut dysbiosis and poor adult health, however, animal studies that also simultaneously assess the impact of mode of deliver would complement these data.

Impact of infant gut colonisation at birth - evidence from germ free mice

Understanding the consequences of different microbial ecologies colonising the infant gut microbiota can be directly investigated using germ-free (GF) animal models i.e. animals lacking microbial colonisation/microbiota. 'Colonisation dysbiosis' appears to occur during critical windows of microbiome development and can result in long-term immunological abnormalities. Chung *et al.* (2012) found that host-specific bacterial species were critical for immune maturation in GF mice. Colonisation of the gut with mouse microbiota at birth normalised the immune phenotype in GF mice to that of wild-type mice. Similarly, GF mice exhibit airway hyper-responsiveness (Herbst *et al.*, 2011) which can be prevented by appropriate microbiota colonisation during the first two weeks of life. This is linked to the formation of 'airway hyper-responsive protective' Helios-Treg cells which are microbiota-dependent within 2 weeks of birth. Further studies would be invaluable to help determine both the microbial components conferring this immune tolerance, their ecological origin, and their human microbiota counterparts.

Altered gut microbiota after CS can impact risk for overweight and obesity, possibly related to the energy harvesting ability of the microbiota. Short chain fatty acids, produced by gut microbiota via anaerobic fermentation are an energy source for colonic epithelial cells and activate G-coupled protein receptors, such as free fatty acid receptor 3 (FFAR3 or Gpr41) which can modulate energy harvest. Experimentally, GF Gpr41^{-/-} mice show increased weight gain on standard diet compared to wild type (Samuel *et al.*, 2008) which could be prevented by specific gut bacteria inoculation. Importantly, Martinez *et al.* (2017) compared weight gain and gut microbiota in mice born by CS (n=34) to vaginally delivered control mice (n=35) and found that the bacterial taxa associated with VD conferred a lean phenotype.

Gut dysbiosis is also been linked to the development of hypertension (Adnan *et al.*, 2017; Li *et al.*, 2017). Li *et al.* (2017) found that the gut microbiota of pre-hypertensive and hypertensive individuals was enriched in *Prevotella*, which favours the development of a pro-inflammatory environment. This low-grade inflammation, triggered by LPS in gram negative bacteria, is thought to be responsible for the pathophysiology of hypertension in these subjects. The authors also demonstrated that hypertension could be transferred to GF mice after a faecal transplant from a human hypertensive donor (Li *et al.*, 2017).

While the relation between the gut peptides and central nervous system has long been recognised, recent evidence from animal studies indicates that the gut microbiota itself may also influence brain function and development (Farmer *et al.*, 2014). Sudo *et al.* (2004) found that GF mice had reduced expression of cortical and hippocampal brain-derived neurotrophic factor (BDNF), a protein involved in neuronal growth and survival, low levels of which has been linked with anxiety-like conditions (Sudo *et al.*, 2004; Bercik *et al.*, 2011; Farmer *et al.*, 2014).

Overall these germ-free models highlight a clear mechanistic pathway from dysbiosis to a range of adverse outcomes, the specific contribution of mode of delivery as causal requires further elucidation.

Effect of Perinatal Antibiotics

The increase in prophylactic antibiotic use during surgery may impact development of infant microbiota (Azad *et al.*, 2016; Martinez *et al.*, 2017). Intrapartum antibiotics can lead to increased abundance of *Bacteroides* and Enterobacteria and reduction of Bacteroidetes in the neonatal gut (Li *et al.*, 2017). Others have also observed an altered gut composition in infants who had been exposed to antibiotics, with reduced diversity and richness of the gut microbiota immediately after birth and impaired the growth of *Clostridiales* and *Ruminococcus* up to 9 months of age (Bokulich *et al.*, 2016).

Mode of Birth and Epigenetic Modification of Gene Expression

Epigenetics offers a molecular mechanism and theoretical construct whereby parturition can impact on gene expression, potentially in a stable and heritable manner, to programme phenotypic traits. DNA methylation is the most commonly studied of the epigenetic mechanisms and in general terms, hypermethylation can effectively silence or attenuate gene expression and hypomethylation ensure genes remain 'switched on'.

According to the emerging EPIIC hypothesis, 'eustress', a healthy stress experienced in normal delivery, is thought to be responsible for priming the fetus for optimal responses in extrauterine life. The stress triggers epigenetic modification of certain genes linked to immune responses, weight control and specific tumour suppressor genes. 'Dys-stress', on the other hand, is a stress response that is either depressed below or elevated above a physiological eustress norm (Dahlen *et al.*, 2013) proposed to affect normal epigenetic processes.

However, it is not yet clear how the events during parturition (use of synthetic oxytocin, antibiotics, and caesarean section) would affect the epigenetic remodelling and subsequent health of the mother and offspring. Behavioural exposures e.g. stress could activate signalling pathways in the brain, which through classical membrane receptor signalling cascades can target histone acetyltransferase, for example (Gabory *et al.*, 2009). It is also likely that intrapartum antibiotics and anaesthetic drugs used during CS may indirectly lead to epigenetic changes through altering the composition of the commensal organisms in both mother and baby.

There are limited studies of mode of delivery and epigenetic changes and those that exist present contradictory findings. One reported global DNA methylation in white blood cells obtained from cord blood was elevated in newborns born by CS without labour compared with those born by vaginal delivery with labour (Schlinzig *et al.*, 2009), the other no evidence of global methylation changes associated with CS versus VD (Virani *et al.*, 2012) despite a larger sample size. Methodological differences in methylation assay and adjustment for maternal factors may account for the disparity between these two studies. In a third study, cord blood samples from babies born via VD (n=37) and CS (n=27), higher levels of global methylation was observed in DNA from CD34+ cells of CS infants (Almgren *et al.*, 2014).

Thus, it is plausible that epigenetic effects of birth have implications in development of the immune system.

Experimental studies employing neonatal stress paradigms have also demonstrated epigenetic alterations in DNA methylation of glucocorticoid receptors in the adult hippocampus associated with increased stress reactivity in later life (Weaver *et al.*, 2004). Furthermore, exposure to synthetic oxytocin, used to induce and augment labour, has been shown to influence feeding cues and can lead to difficulties with breastfeeding by an unknown mechanism (Bell *et al.*, 2013; Marín Gabriel *et al.*, 2015). Dahlen *et al.* suggest that these effects of synthetic oxytocin are modulated by epigenetic remodelling in both mother and infant (Dahlen *et al.*, 2013). The functional correlates of any ‘parturition’ associated global methylation status in cord blood and persistence into childhood and beyond requires further follow-up.

More numerous and larger human studies are needed to assess the impact of mode of delivery on epigenetic mechanisms and long-term biological implications. Developing appropriate animal models would help in this regard.

What can be done to mitigate the effects of CS?

Optimisation of childbirth

Ideally, the negative impact of mode of delivery could be avoided by promoting normal physiological processes during childbirth in an appropriate and safe environment.

Assessment, care planning, health education, and promotion of normal processes could set the stage for strengthening the woman’s capabilities in preparation for the labour, birth, and motherhood (Renfrew *et al.*, 2014). Helping women understand the physiological value of awaiting spontaneous labour and to prepare to cope with the rigors of labour can foster eustress and support the interconnection of the complex biological and chemical processes to benefit the mother and newborn. Several processes can be supported by clinical care givers in this regard including: i) Minimization of fear and anxiety: Most mammals will only give birth in situations where they feel safe and this has implications for human childbirth.

Feeling safe may account for robust evidence from Cochrane Reviews on the effectiveness of midwife-led continuity of care, alternative birth settings, and continuous support in labour on

increased spontaneous vaginal birth (Hodnett *et al.*, 2012; Sandall *et al.*, 2016; Bohren *et al.*, 2017). Reciprocal knowledge and trust in the woman-provider relationship may be one of many factors to improved physiological labour functioning and avoidance of CS; ii) Organisational procedures could reduce unnecessary caesarean section through implementation of appropriate guidelines (Khunpradit *et al.*, 2011; National Institute for Health and Care Excellence, 2017). A study of 2400 US mothers' experience of childbirth found that women felt pressured to have certain interventions such as labour induction (25%), primary CS (28%), and repeat CS (22%) (Declercq *et al.*, 2013). Expectant management and avoiding interventions unless clinically indicated, can avoid the use of synthetic oxytocin, averting uterine hyperstimulation, increased pain, and disruption of the maternal and fetal/newborn regulation that promotes breastfeeding (prolactin), attachment, and maternal mood postpartum (Buckley, 2015). All interventions must be evaluated from a "first, do not harm" perspective. Simply because a CS is a relatively safe procedure, potential long-term consequences cannot be ignored (Kennedy *et al.*, 2016).

However, there will always be critical medical indications for assisted delivery and CS, currently approximately 10%, but increasing with advancing maternal age and the obesity epidemic. Thus, efforts should be made to keep CS as physiological as possible to evoke normal developmental cues. One such method of maintaining the physiological integrity of normal delivery could be to mimic the surge of hormones associated with VD. As identified above, infants delivered by CS can have an increased risk of respiratory complications because of reduced exposure to cortisol during delivery (Jain & Dudell, 2006). Promising results from randomised trials show that administration of antenatal steroids to mothers delivering full term infants by elective CS improved neonatal respiratory outcomes (Stutchfield, 2005). Furthermore, a systematic review, including three RCTs of full term infants delivered by elective CS, found that administering antenatal steroids 48 hours prior to delivery reduced the risk of respiratory distress syndrome, transient tachypnoea, and reduced the length of stay in the NCU (Saccone & Berghella, 2016). However, the use of corticosteroids steroids is controversial and would have to be very carefully reviewed before being implemented on a widescale.

An alternative method of mimicking the effects of normal delivery that may be more acceptable is ensuring skin to skin contact at delivery. Skin to skin contact, resembling the Madigan squeeze technique, was administered to 39 full term neonates born via CS and VD

during the first two days of life. A dose response relationship was observed and a reduction in the levels of inhibitory neurosteroids (McCallie *et al.*, 2017). Although this effect was only significant for pregnanolone in all deliveries, plus progesterone in infants delivered via VD, the authors suggested that a different response may be observed in critically ill or preterm infants (McCallie *et al.*, 2017).

Vaginal seeding

Vaginal seeding is a relatively new practice, but is increasing in popularity. This technique involves a sterile gauze being incubated inside the vagina and applied to the neonate's skin to inoculate with maternal bacteria they would have otherwise been exposed to during a vaginal delivery (Dominguez-Bello *et al.*, 2016). However, there is limited evidence on safety of this practice and its efficacy in improving infant health in the long term (Cunnington *et al.*, 2016; Dominguez-Bello *et al.*, 2016). One important consideration when carrying out this technique is the risk of infections, particularly *Streptococcus B* (Cunnington *et al.*, 2016). However, as discussed above, the limited evidence indicating that the vaginal microbiota is responsible for seeding the gut indicates that facilitation of maternal faecal seeding or probiotic intervention should also be considered.

Breastfeeding

Breastfeeding contributes to colonisation of the gut, and strongly influences the seeding of the gut, particularly in those born by CS (Hill *et al.*, 2017). Human milk oligosaccharides (HMO) are the third most abundant component of breastmilk which supports *B.infantis* growth (Underwood *et al.*, 2015) which is particularly important for infants born via CS due to their low abundance of *Bifidobacterium* (Frese *et al.*, 2017). Infant formula, which has been found to alter the normal development of gut microbiota in both VD infants and those delivered via CS (Yasmin *et al.*, 2017), does not contain these essential HMOs. The INFANTMET cohort examined the evolution of gut microbiota in full term and preterm infants born by CS and VD and found that at 4 weeks old the gut microbiota of breastfed infants born via CS begins to resemble that of an infant delivered vaginally, with an increased abundance of Actinobacteria (Hill *et al.*, 2017), and this resemblance strengthened from weeks 4 to 8 with an increase in Bacteroidetes in CS infants. This highlights the importance of breastfeeding in establishing a "normal" gut microbiota in infants born via CS. As women

who deliver via CS have increased rates of breastfeeding problems and early cessation, particularly those who have an elective CS, one pathway to optimising the health of the infant would to consistently provide women delivering via CS with more support to breastfeed.

Supplementation of probiotics

Given the role gut microbiota plays in the development of the immune system, reversing dysbiosis could potentially improve long-term outcome for CS infants, especially if undertaken in parallel with breast feeding.

Animal studies have demonstrated the potential use of probiotic supplementation of neonates to improve immune function (Lewis *et al.*, 2017; Underwood *et al.*, 2014). In human neonates, supplementation of the probiotic *B. infantis* (strain EVC001, IMPRINT Study) for 21 days appears to be tolerated in breastfed babies. Supplementation significantly increased the level of *B. infantis* in infant fecal samples compared to the control (Smilowitz *et al.*, 2017); an effect that persisted for more than 30 days after supplementation. *B. infantis* supplementation also led to an increase in faecal acetate and lactate, decreases in faecal pH and bacterial endotoxin and fewer stools, all indications of improved microbiome (Frese *et al.*, 2017). Whilst mode of delivery was not the focus of this study, babies from CS deliveries generally had less *B. infantis* than VD babies and this was reversed by probiotic treatment. A larger study of CS infants including child health and gut follow up would be useful.

Conclusions

There is a growing body of evidence that mode of delivery, particularly pre-labour CS can have long term health consequences for the infant. There is still a need to experimentally establish a direct causal pathway from mode of delivery via microbiome alterations to long term health issues, but observational evidence is convincing. Increased efforts to reduce CS rates *per se* as well implementing care pathways that optimise parturition physiology are required. There seems promise in pursuing interventions that promote neonatal gut health and breastfeeding. The impact of epigenetic changes imposed by mode of delivery is an interesting concept, but requires more convincing experimental evidence.

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Additional Information Section

Competing Interests

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Abstract Diagram: The different experiences of babies born vaginally and by caesarean section impacts several developmental and physiological processes which in turn influence short and long-term health.

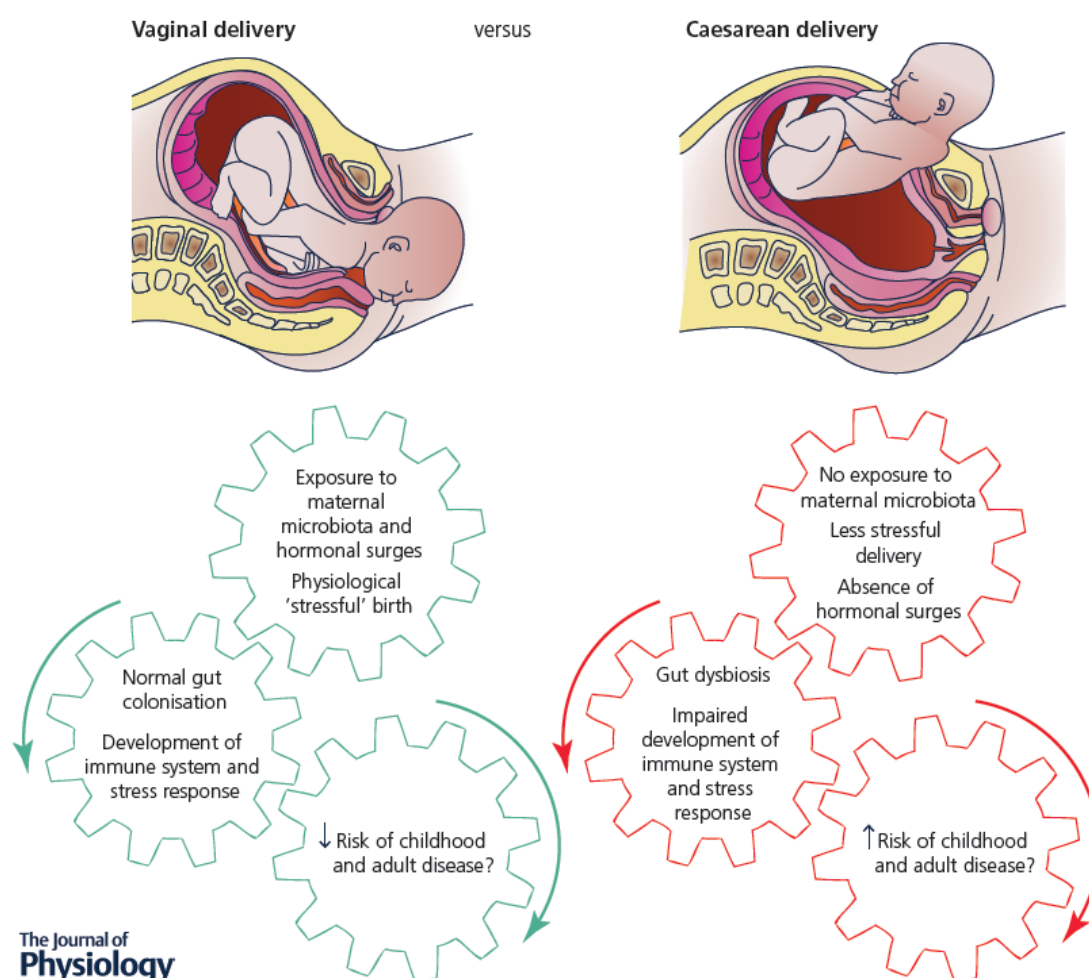


Figure 1. Exposures that contribute to the colonisation of the gut and how the gut impacts health

